

PCTWORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : A23L 3/3571	A1	(11) International Publication Number: WO 96/31133 (43) International Publication Date: 10 October 1996 (10.10.96)
(21) International Application Number: PCT/DK96/00161 (22) International Filing Date: 3 April 1996 (03.04.96) (30) Priority Data: 0400/95 7 April 1995 (07.04.95) DK (71) Applicant (for all designated States except US): NOVO NORDISK A/S [DK/DK]; Novo Allé, DK-2880 Bagsværd (DK). (72) Inventors; and (75) Inventors/Applicants (for US only): PETERSEN, Bent, Riber [DK/DK]; Novo Nordisk A/S, Novo Allé, DK-2880 Bagsværd (DK). MATHIASSEN, Thomas, Erik [DK/DK]; Novo Nordisk A/S, Novo Allé, DK-2880 Bagsværd (DK). (74) Common Representative: NOVO NORDISK A/S; Corporate Patents, Novo Allé, DK-2880 Bagsværd (DK).		(81) Designated States: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i>
(54) Title: DEOXYGENATION OF A FOOD ITEM USING A LACCASE (57) Abstract A simple and effective method of deoxygenating a food item derived partly or wholly from an extract of a plant material by use of a laccase. The food item may be a juice, a soup or a concentrate.		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia	GB	United Kingdom	MW	Malawi
AT	Austria	GE	Georgia	MX	Mexico
AU	Australia	GN	Guinea	NE	Niger
BB	Barbados	GR	Greece	NL	Netherlands
BE	Belgium	HU	Hungary	NO	Norway
BF	Burkina Faso	IE	Ireland	NZ	New Zealand
BG	Bulgaria	IT	Italy	PL	Poland
BJ	Benin	JP	Japan	PT	Portugal
BR	Brazil	KE	Kenya	RO	Romania
BY	Belarus	KG	Kyrgyzstan	RU	Russian Federation
CA	Canada	KP	Democratic People's Republic of Korea	SD	Sudan
CF	Central African Republic			SE	Sweden
CG	Congo	KR	Republic of Korea	SG	Singapore
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
CI	Côte d'Ivoire	LI	Liechtenstein	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LR	Liberia	SZ	Swaziland
CS	Czechoslovakia	LT	Lithuania	TD	Chad
CZ	Czech Republic	LU	Luxembourg	TG	Togo
DE	Germany	LV	Latvia	TJ	Tajikistan
DK	Denmark	MC	Monaco	TT	Trinidad and Tobago
EE	Estonia	MD	Republic of Moldova	UA	Ukraine
ES	Spain	MG	Madagascar	UG	Uganda
FI	Finland	ML	Mali	US	United States of America
FR	France	MN	Mongolia	UZ	Uzbekistan
GA	Gabon	MR	Mauritania	VN	Viet Nam

DEOXYGENATION OF A FOOD ITEM USING A LACCASE

FIELD OF INVENTION

This invention relates to a simple and effective method of deoxygenating food items derived partly or wholly from extracts of plant materials. The food items may be juice, soup or concentrates.

BACKGROUND OF THE INVENTION

Pasteurized and unpasteurized citrus juices lose some of their typical quality during processing and storage as a result of temperature, dissolved oxygen and storage time. The influence of oxygen on the quality of pasteurized citrus juices has been investigated extensively and it has been shown that oxygen takes part in the degradation of ascorbic acid and also affects browning of the juices. Oxygen has also a damaging effect on some of the aroma components and may decrease their strength (for reference see Journal of Food Processing and Preservation 14, 1990, pp. 253-256).

In the tomato industry the content of dissolved oxygen should also be minimized as oxygen may take part in a discolouring process of the tomato products, e.g., produce a "Black neck" in bottled catsup, which is a dark discolouration of the top layer presumably due to oxidation (for reference see "Tomato Production, Processing & Technology", 3rd Ed., CTI Publications Inc., p. 298 and p. 240).

Enzymatic deoxygenation with glucose oxidase has been described to reduce the amount of dissolved oxygen in juices significantly (for reference see Journal of Food Processing and Preservation 14, 1990, pp. 253-256). Glucose oxidase catalyzes the oxidation of d-glucose to d-gluconic acid in the presence of molecular oxygen: $C_6H_{12}O_6 + 2O_2 + 2H_2O \rightarrow 2C_6H_{12}O_7 + 2H_2O_2$. As it can be seen, a by product of this reaction is H_2O_2 , which can be destructive to juice constituents. In order to avoid this problem it has been suggested to add a

catalase together with the glucose oxidase; catalase catalyzes the reaction: $2\text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{O}_2$, but then oxygen is produced again! - however, the overall effect of the enzymatic deaeration by using a combination of glucose oxidase and a catalase is removal of 0.5 mole oxygen for each mole of oxidized d-glucose.

It is an object of the present invention to find a simple and effective enzyme system for oxygen removal in food items derived partly or wholly from extracts of plant materials. The food item may be a juice, a soup or a concentrate.

SUMMARY OF THE INVENTION

It has surprisingly been found that laccases are very efficient in reducing the oxygen content in for example a tomato soup.

Accordingly, the present invention provides a method of deoxygenating a food item derived partly or wholly from an extract of a plant material, the method comprising adding an effective amount of a laccase to said food item and/or to said extract.

BRIEF DESCRIPTION OF DRAWINGS

The present invention is further illustrated by reference to the accompanying drawings, in which

Fig. 1 shows the relation between % oxygen and time in a tomato soup which is first aerated whereafter a laccase is added, the experiment conducted as described in Example 1.

Fig. 2 shows the relation between % oxygen and time in a wheat gluten hydrolysate after a laccase is added, the experiment conducted as described in Example 2.

DETAILED DISCLOSURE OF THE INVENTION

According to the present invention a food item may be deoxygenated by adding an effective amount of a laccase.

Preferred food items are those which are partly or wholly derived from an extract of a plant material.

5 According to the invention the food item may be anything eatable or drinkable comprising an extract of a plant material, e.g., a juice, a soup, or a concentrate. Concentrates may be in the form of a pulp, a puree, a catsup or a paste.

In the context of this invention an extract from a
10 plant material is any substance which can be derived from plant material by extraction, processing or by other separation techniques.

The extract may be provided on the basis of frozen materials, heat treated materials, fresh materials, or provided
15 in any other form known in the art. The extract may contain the plant material, e.g., the fruit, in the form as whole fruits, chopped fruits, or the fruits may be mashed, pulped or homogenized.

Useful extracts may also be in the form of hydro-
20 lysates, in particular protein hydrolysates; these protein hydrolysates may be prepared, e.g., as described in "Enzymic Hydrolysis of Food Proteins", written by Jens Adler-Nissen, Elsevier Applied Science Publishers LTD, 1986.

Examples of such protein hydrolysates are wheat
25 gluten and maize gluten (corn gluten), wherein the wheat gluten is a highly valued functional protein and maize gluten, which is a by-product from the production of starch from maize, is a highly valued feed protein due to its content of xanthophyll.

Other examples of such protein hydrolysates are
30 plant proteins derived from oilseeds or beans, e.g., cottonseed, peanuts, sesame, sunflower, rapeseed, or the Vicia faba bean (field bean, broad bean, horse bean, faba bean).

The plant protein may also be derived from potatoes; this protein is available in large quantities as a by-product
35 from the production of potato starch, where it may be recovered by e.g. heat coagulation.

According to the present invention the plant material may be obtainable from e.g. fruits, vegetables, cereals, oilseeds or beans.

Useful fruits are, e.g., pome or seed fruits (apples, pears, etc.), grapes, tomatoes, citrus (orange, lemon, lime, mandarin), prunes, cherries, black currants, red currants, raspberries, strawberries, cranberries, pineapple, or any other tropical fruit.

Useful vegetables are, e.g., potatoes, carrots, celery, or onions.

Useful cereals are, e.g., wheat or maize.

Useful oilseeds are, e.g., cottonseed, peanuts, sesame, sunflower or rapeseed.

A useful bean may be the Vicia faba bean.

The plant material may also contain more than one fruit, or more than one vegetable, or more than one cereal, etc., or it may contain a mixture of, e.g., a fruit and a vegetable.

According to the present invention especially food items derived from tomatoes or citrus are preferred, in particular tomato products such as juices, soups or concentrates, and citrus products such as juices, in particular orange juice and lemon juice.

Food items such as juice, puree and catsup are defined and produced in a manner known per se, e.g., as defined by the Food & Drug Administration:

The Food & Drug Administration defines tomato juice, puree/pulp and catsup in the following way (for reference see "Tomato Production, Processing & Technology", 3rd Ed., CTI Publications Inc., Appendix B, Part 53):

Tomato juice: Tomato juice is the unconcentrated liquid extracted from mature tomatoes of red or reddish varieties, with or without scalding followed by draining. In the extraction of such liquid, heat may be applied by any method which does not add water thereto. Such liquid is strained free from skins, seeds, and other coarse or hard substances, but carries finely divided insoluble solids from

the flesh of the tomato. Such liquid may be homogenized, and may be seasoned with salt. When sealed in a container it is so processed by heat, before or after sealing.

Tomato puree/pulp: Tomato puree, tomato pulp, is the food prepared from one or any combination of two or all of the following optional ingredient:

(1) The liquid obtained from mature tomatoes of red or reddish varieties.

(2) The liquid obtained from the residue from preparing such tomatoes for canning, consisting of peelings and cores with or without such tomatoes or pieces thereof.

(3) The liquid obtained from the residue from partial extraction of juice from such tomatoes.

(4) Salt.

Catsup: Catsup, ketchup, catchup, is the food prepared from one or any combination of two or all of the following optional ingredient:

(1) The liquid obtained from mature tomatoes of red or reddish varieties.

(2) The liquid obtained from the residue from preparing such tomatoes for canning, consisting of peelings and cores with or without such tomatoes or pieces thereof.

(3) The liquid obtained from the residue from partial extraction of juice from such tomatoes.

Phenolic compounds

According to the present invention, depending on the food item in question, it may in some cases be an advantage also to add a phenolic compound, in which case the phenolic compound acts as a substrate for the laccase, thereby increasing the deoxygenation of said food item.

Phenolic compounds which may be used according to the invention, could be an anthocyanin or a spice such as paprika. Of course the phenolic compound used should be one normally allowable as a food ingredient.

Laccase

Laccase (EC 1.10.3.2) is characterized by being a group of multi-copper proteins of low specificity acting on both o- and p-quinols, whereby oxygen is reduced to water.

According to the invention microbial laccase is preferred. The microbial laccase may be derived from bacteria or fungi (including filamentous fungi and yeasts). The microbial laccase is preferably obtained from a fungus.

Some preferred fungi include strains belonging to the subdivision Basidiomycotina and to the subdivision Ascomycotina. Suitable examples include a laccase derivable from a strain of Aspergillus, Neurospora, e.g., N. crassa, Podospora, Botrytis, Collybia, Fomes, Lentinus, Pleurotus, Trametes, e.g., T. villosa and T. versicolor, Rhizoctonia, e.g., R. solani, Coprinus, e.g., C. plicatilis and C. cinereus, Psatyrella, Myceliophthora, e.g., M. thermophila, Schytalidium, e.g., S. thermophilum, Polyporus, e.g., P. pinsitus, Phlebia, e.g., P. radita (WO 92/01046), Coriolus, e.g., C. hirsutus (JP 2-238885), Hygrophoropsis, Agaricus, Vascellum, Crucibulum, Myrothecium, or Sporormiella.

In particular laccases derivable from T. villosa, T.versicolor, M. thermophila or S. thermophilum are preferred.

The laccase may furthermore be one which is producible by a method comprising cultivating a host cell transformed with a recombinant DNA vector which carries a DNA sequence encoding said laccase as well as DNA sequences encoding functions permitting the expression of the DNA sequence encoding the laccase in a culture medium under conditions permitting the expression of the laccase and recovering the laccase from the culture.

Determination of Laccase Activity (LACU)

Laccase activity is determined from the oxidation of 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonate) (ABTS) by oxygen. The greenish-blue colour produced is photometered at 418 nm. The analytical conditions are 1.67 mM ABTS, 0.1 M phosphate buffer, pH 7.0, 30°C, 3 minutes reaction.

1 laccase unit (LACU) is the amount of enzyme that catalyses the conversion of 1 μ mole ABTS per minute at these conditions.

Addition of Laccase

5 The food items according to this invention may be produced in the manner known per se.

According to the invention addition of laccase may take place at any step during the manufacture of said food item, often as the last or one of the last processing steps, 10 but laccase may also be added as a prophylactic treatment at an earlier stage in order to remove any oxygen that may come into the food item during processing, or the laccase may be added twice: first as a prophylactic treatment and then in one of the last processing steps to ensure that any oxygen left in the 15 food item is effectively removed.

The amount of laccase added will typically be in the range of from 0.01-100 LACU per g dry matter of the food item, preferably in the range of from 0.1-5 LACU per g dry matter of the food item.

20 The invention is further illustrated in the following examples which are not intended to be in any way limiting to the scope of the invention as claimed.

EXAMPLE 1

Deoxygenation of Tomato Soup with Laccase.

25 The percentage of oxygen in a commercially available tomato soup (Heinz) was measured by means of a Mettler Toledo Oxygen Electrode. The result is presented in Fig. 1.

It can be seen that the oxygen concentration in this soup was only a few per cent of saturation. The soup was then 30 aerated for 10 minutes to a level of approximately 90% saturation (cf. Fig. 1).

A laccase (a Trametes villosa laccase available from Novo Nordisk A/S under the trade name SP 504) at a dosage of 3.5 LACU per g dry matter (soup) was now added under continuous

stirring. Temperature was ambient (25°C) and pH was measured to 4.6.

As shown in Fig. 1 laccase is clearly able to deoxygenate the tomato product as the oxygen level is reduced from about 80% to about 60% in approximately 7 minutes.

EXAMPLE 2

Deoxygenation of a Solution of Wheat Gluten Hydrolysate.

30.36 g of wheat gluten hydrolysate was dissolved in 2.15 l of deionized water at 30.5°C. pH was adjusted to 4.5.

10 3 LACU of a laccase (a Trametes villosa laccase available from Novo Nordisk A/S under the trade name SP 504) per g of wheat gluten hydrolysate was added, and the oxygen concentration was followed for 40 minutes.

At 30.5°C the oxygen content in 2.15 l of water is 15 approximately 2.5 mmoles which was defined as 100%. The enclosed graph (Fig. 2) showing oxygen consumption versus time indicates a degree of deoxygenation corresponding to 0.000013 moles of O₂ per g of wheat gluten hydrolysate in 40 minutes.

CLAIMS

1. A method of deoxygenating a food item derived partly or wholly from an extract of a plant material, comprising adding an effective amount of a laccase to said food item
5 and/or to said extract.

2. A method according to claim 1, wherein the food item is a juice, a soup or a concentrate.

3. A method according to claim 2, wherein the concentrate is a puree, a catsup or a paste.

10 4. A method according to claim 1, wherein the plant material is obtainable from fruits, vegetables or cereals.

5. A method according to claim 4, wherein the fruits are selected from the group consisting of apples, pears, citrus, tomatoes, grapes, black currants, red currants,
15 raspberries, strawberries, cranberries, prunes, cherries and pineapple.

6. A method according to claim 5, wherein the citrus are selected from the group consisting of oranges, lemons, lime and mandarins.

20 7. A method according to claim 4, wherein the vegetables are selected from the group consisting of potatoes, carrots, celery and onions.

8. A method according to claim 4, wherein the
25 cereals are selected from the group consisting of wheat and maize.

9. A method according to claim 1, wherein the extract is a protein hydrolysate.

10. A method according to claim 9, wherein the protein hydrolysate is derived from a cereal.

11. A method according to claim 1, wherein the laccase is a microbial laccase.

5 12. A method according to claim 11, wherein the microbial laccase is derivable from a fungus, in particular from a fungus belonging to the subdivision Basidiomycotina or to the subdivision Ascomycotina.

10 13. A method according to claim 12, wherein the microbial laccase is derivable from a strain of Aspergillus, Neurospora, Podospora, Botrytis, Collybia, Fomes, Lentinus, Pleurotus, Trametes, Rhizoctonia, Coprinus, Psatyrella, Myceliophthora, Schytalidium, Polyporus, Phlebia, Coriolus, Hygrophoropsis, Agaricus, Vascellum, Crucibulum, Myrothecium,
15 or Sporormiella.

14. A method according to claim 13, wherein the microbial laccase is derivable from T. villosa, T. versicolor, M. thermophila or S. thermophilum.

20 15. A method according to any of claims 1-14, wherein the amount of laccase is in the range of from 0.01-100 LACU per g dry matter of the food item, preferably in the range of from 0.1-5 LACU per g dry matter of the food item.

16. A method according to claim 1, additionally adding a phenolic compound.

1/2

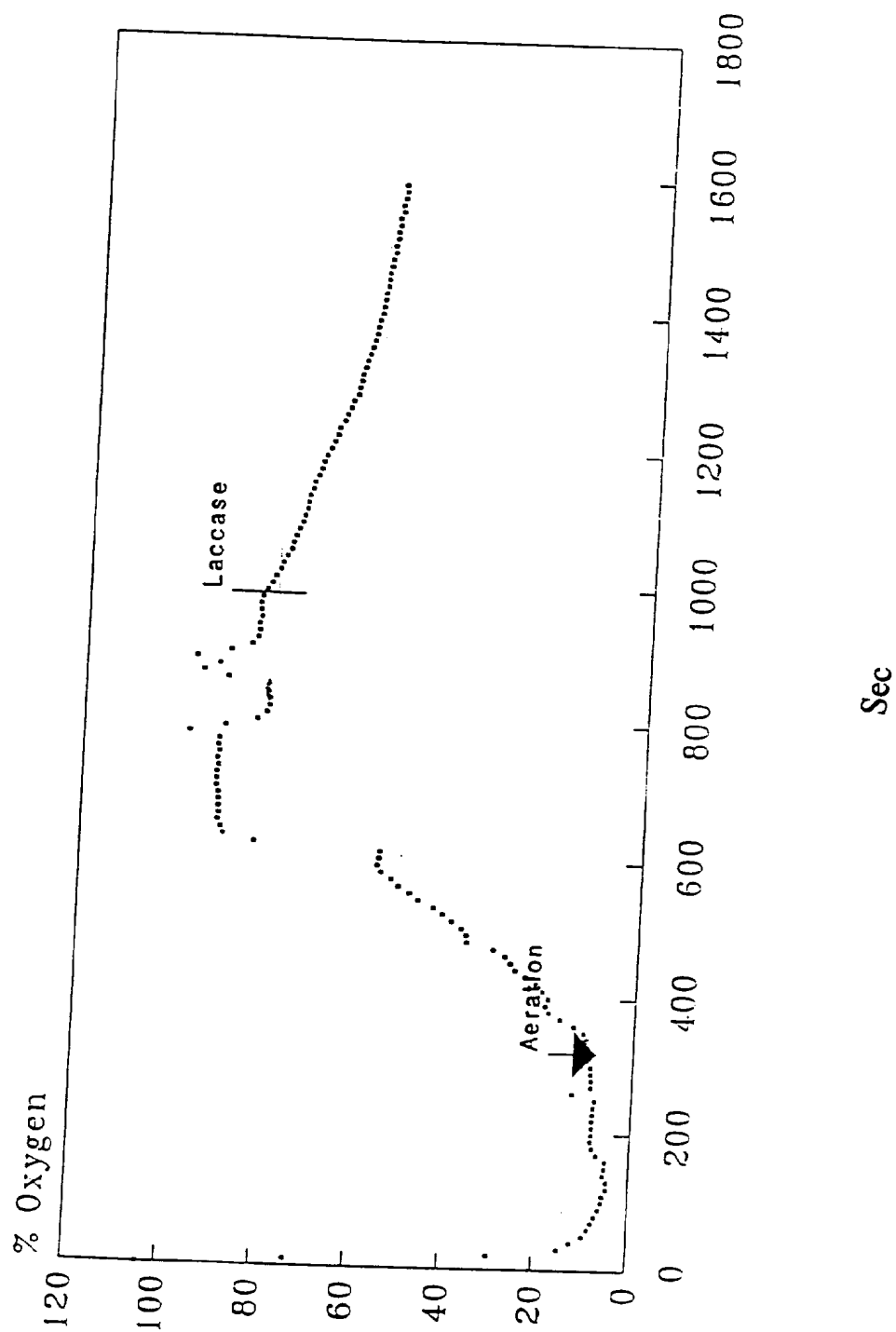


Fig. 1

2/2

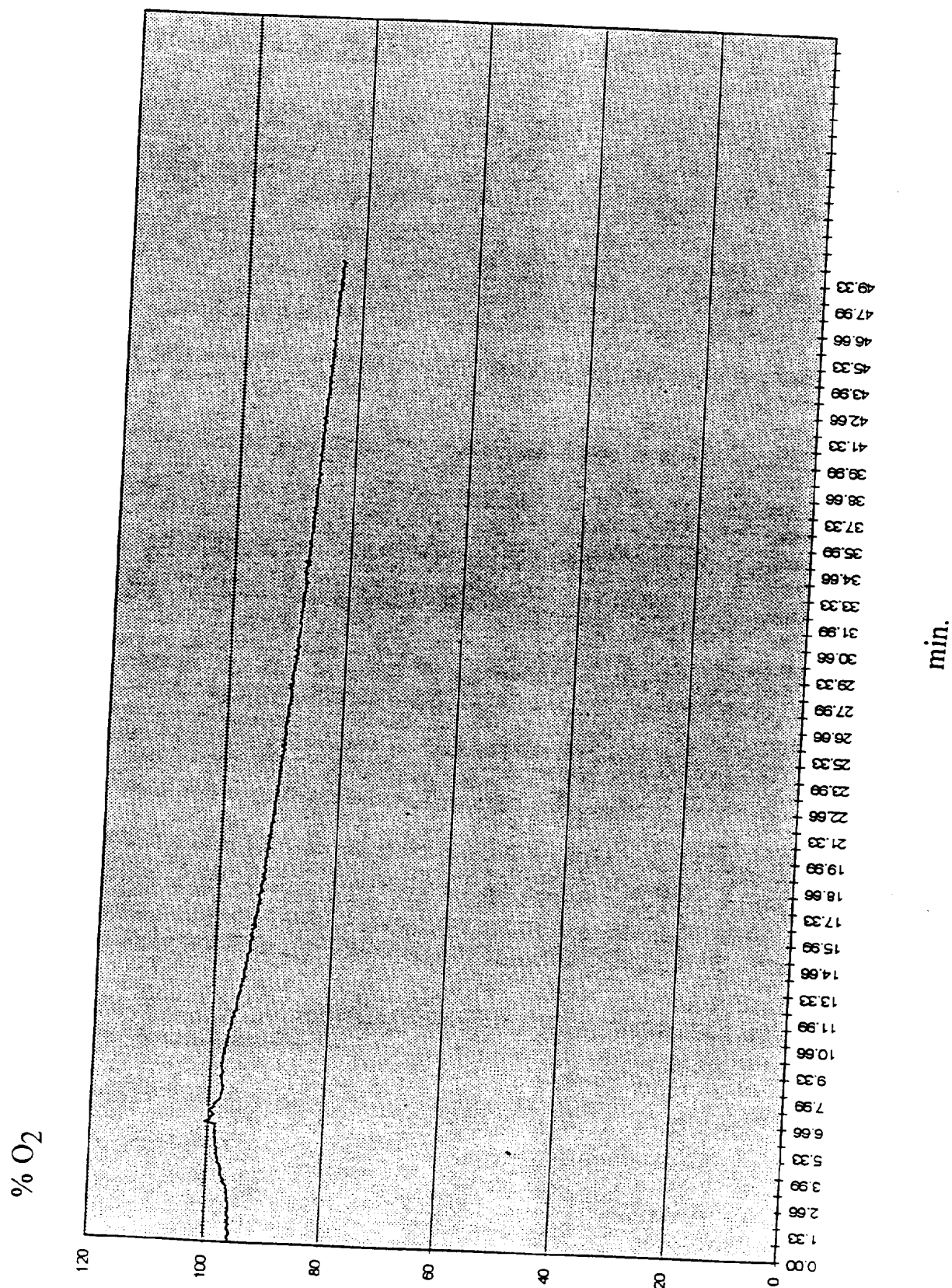


Fig. 2

INTERNATIONAL SEARCH REPORT

International application No.

PCT/DK 96/00161

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: A23L 3/3571

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: A23L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI, CLAIMS/US PATENTS, JAPIO, DIALINDEX(FOODSCI)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5180672 A (H. ITOH ET AL.), 19 January 1993 (19.01.93), abstract --	1
A	EP 0304029 A1 (SUNTORY LIMITED), 22 February 1989 (22.02.89), claim 6 --	1
A	DE 3204284 A1 (G. KOHLBECKER), 18 August 1983 (18.08.83), abstract -- -----	1

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

3 June 1996

Date of mailing of the international search report

04 -06- 1996

Name and mailing address of the ISA/
Swedish Patent Office

Box 5055, S-102 42 STOCKHOLM

Facsimile No. +46 8 666 02 86

Authorized officer

INGA-KARIN PETERSSON

Telephone No. +46 8 782 25 00

INTERNATIONAL SEARCH REPORT

Information on patent family members

01/04/96

International application No.

PCT/DK 96/00161

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US-A- 5180672	19/01/93	EP-A, A, A 0442781 JP-A- 3236766	21/08/91 22/10/91
EP-A1- 0304029	22/02/89	DE-A- 3866630 JP-A- 1047364 US-A- 4867990	16/01/92 21/02/89 19/09/89
DE-A1- 3204284	18/08/83	NONE	